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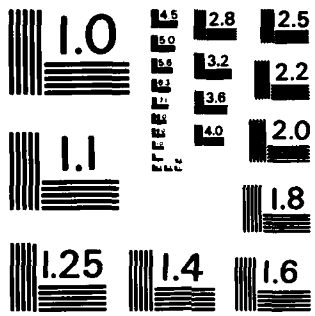

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DETERMINATION OF THE TOXICITY TO AQUATIC ORGANISMS OF HMX AND RELATED WASTEWATER CONSTITUENTS

PART 2:

THE ACUTE AND CHRONIC TOXICITY OF ACETONE, DIMETHYL FORMAMIDE, AND TRIETHYLENE GLYCOL TO *Daphnia magna* (Straus).

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Gerald A. LeBlanc
Donald C. Surprenant
Robert E. Bentley
and
Sam R. Petrocelli

EG & G Bionomics, Aquatic Toxicology Laboratory, Wareham, MA

January, 1983

Final Report

Contract No. DAMD 17-80-C-0011

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Prepared for

Health Effects Division
U.S. ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

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Acute and chronic toxicity tests were performed with three organic solvents commonly used to facilitate the solubilization of lipophilic compounds during acute toxicity tests with *Daphnia magna* (Straus). The 48-hour LC50 values and corresponding 95% confidence intervals were: acetone, 39000(31000-53000) µL/L; dimethyl formamide, 13000(10000-16000) µL/L; triethylene glycol, 35000(28000-46000) µL/L. Maximum acceptable toxicant concentra-

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FOREWORD

Citation of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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EXECUTIVE SUMMARY

Acute and chronic toxicity tests were performed with three organic solvents commonly used to facilitate the solubilization of lipophilic compounds during aquatic toxicity tests with <u>Daphnia magna</u> (Straus). The 48-hour LC50 values and corresponding 95% confidence intervals were: acetone, 39000(31000-53000) μL/L; dimethyl formamide, 13000(10000-16000) μL/L; triethylene glycol, 35000(28000-46000) μL/L. Maximum acceptable toxicant concentrations determined during the chronic toxicity tests were: acetone, >1400<2800 μL/L; dimethyl formamide, >1200<2500 μL/L; triethylene glycol, >5500<11000 μL/L. Triethylene glycol was the least chronically toxic solvent and is recommended as the primary choice when selecting a carrier solvent during aquatic toxicity tests. All three solvents were sufficiently low in toxicity to suggest that the recommended usage limits (500 μL/L during acute toxicity tests, 100 μL/L during long-term toxicity tests) are adequate for the prevention of solvent-related toxicity to <u>D. magna</u>.

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INTRODUCTION

The organic solvents acetone, dimethyl formamide and triethylene glycol are commonly used agents for facilitating the solubilization or introduction of lipophilic compounds into water during aquatic toxicity tests. Standardized procedures in aquatic toxicity testing generally recommend that solvents not be used in excess of 500 μL/L (ppm) during acute toxicity tests (U.S. EPA, 1975) and 100 μL/L during longer term toxicity tests (Adams, 1981; Benoit, 1981; Comotto, 1981). Although these criteria were based on existing scientific rationale, there currently exists a paucity of published data evaluating the long-term effects of these compounds on aquatic organisms. Acute lethality data has been generated with these three compounds and fish. Using fathead minnow (*Pimephales promelas* Rafinesque), brook trout (*Salvelinus fontinalis* Mitchill) and bluegill (*Lepomis macrochirus* Rafinesque), Cardwell et al. (1978) determined the 96-hour LC50's for acetone to range from 6,070 to 9,100 mg/L, for dimethyl formamide to range from 6,300 to 10,410 mg/L, and for triethylene glycol to range from 61,000 to 92,500 mg/L. Corroborative data were obtained for acetone by Majeswki (1978) with rainbow trout (*Salmo gairdneri* Richardson) and Smith (1974) with bluegill. Majeswki et al. (1978) estimated the 24-hour LC50 for acetone in the rainbow trout under flow-through conditions to be 6100 mg/L and Smith (1974) estimated the 96-hour LC50 to be 8300 mg/L for bluegill. Statistically significant chronic toxicity of dimethyl formamide and triethylene glycol was detected by Cardwell et al. (1978). These investigators determined the threshold effect concentration for dimethyl formamide to be between

43 and 98 mg/L for brook trout and 5 and 11 mg/L for fathead minnows. Threshold effect concentrations for triethylene glycol were between 21 and 48 mg/L for rainbow trout and 82 to 180 mg/L for fathead minnows. These data suggest that the criterion level of 100 µL/L for dimethyl formamide and triethylene glycol is too high for the conduct of long-term aquatic toxicity tests.

The purpose of this study was to determine the acute and chronic toxicity of acetone, dimethyl formamide and triethylene glycol to the aquatic invertebrate *Daphnia magna* (Straus). *D. magna* was selected as the test organism because this organism is commonly used for chronic toxicity tests and due to the relative dearth of information relating to the toxicity of organic solvents to aquatic invertebrates. Also, the sensitivity of daphnids to toxins tends to be representative of freshwater zooplankton and is correlative to fish sensitivity (Maki, 1979).

MATERIALS AND METHODS

The acetone (lot #9N28) and the dimethyl formamide (lot #11L28B) were obtained from Curtin Matheson Scientific Inc., Boston, Massachusetts. The triethylene glycol (lot #B1A) was purchased from the American Scientific Products Co., McGraw Park, Illinois. All solvents used were of analytical grade.

Dilution water used in these tests consisted of soft well water which was fortified to provide a total hardness and alkalinity as

$CaCO_3$ of 165 ± 15 mg/L and 120 ± 10 mg/L, respectively, a pH of 7.9-8.3 and a specific conductance of 400-600 μmhos/cm. The salts and quantities used to fortify the water were based on the formula for hard water presented by U.S. EPA (1975). The water was filtered through an Amberlite XAD-7 resin column prior to use to remove any potential organic contaminants.

The daphnids used during this study were obtained from laboratory stocks cultured at EG&G Bionomics. Culture water was of the same quality as the dilution water previously mentioned. Daphnids were fed a combination of Rangen Salmon Starter fish food suspension (5 mg/mL) and mixed species unicellular green algae ad libitum. All daphnids used to initiate tests were ≤24 hours old.

Acute Toxicity Tests

The acute toxicity tests were performed in 250-mL beakers containing 150 mL of test solution. Test solutions of the solvents were prepared by adding the desired volume of solvent to 500 mL of dilution water and mixing briefly on a magnetic stirrer. Four hundred and fifty milliliters of each solution were then divided into three beakers to provide replicate exposure treatments. Triplicate controls consisting of the same dilution water and maintained under the same conditions as the solvent solutions, but containing no solvent, were established for each test. The solution temperatures were maintained at 21 ± 1°C by ambient room temperature. Test solutions were not aerated. The test area was illuminated with Durotest (Optima 50) fluorescent lights at an intensity of 100-150 footcandles.

Fifteen daphnids were impartially distributed to each treatment level (5 per replicate beaker) within 30 minutes after the test solutions had been prepared. Daphnids were not fed during exposure. Mortalities and behavioral abnormalities observed in the replicate test solutions were recorded at 0, 24 and 48 hours of exposure. The pH's and dissolved oxygen concentrations were measured at 0 and 48 hours exposure in one replicate of the control and the high middle and low test concentrations. The temperature was measured at 0, 24 and 48 hours in one replicate of the control. The mortality data derived from the acute toxicity tests were used to estimate 24- and 48-hour median lethal concentrations (LC50) and 95% confidence intervals. The LC50 values were calculated by the moving average angle method if two or more partial responses were observed. Binomial probability was used if only one partial response was observed.

Chronic Toxicity Tests

Results of the acute toxicity tests were used to select the concentration for the chronic toxicity tests. Two hundred milliliter proportional diluters (Mount and Brungs, 1967), calibrated to provide 50 percent dilutions, delivered the solvent solutions to the test aquaria during the chronic toxicity tests. Each diluter was equipped with a tube-siphon delivery apparatus (Figure 1) which introduced the desired amount of solvent into the mixing chamber of the diluter with every diluter cycle. Aquaria were glass battery jars with a volume capacity of 1.75 liters. Test solutions drained from the aquaria through a 3 x 8 cm notch cut

on the upper edge of the jars. Notches were covered with a Nitex[R] 40-mesh screen to prevent loss of the daphnids. Five concentrations of each solvent were assessed in addition to a set of controls which were maintained with each test. All treatments and controls were quadruplicated. Test solutions were delivered to the aquaria at a rate of 4 to 5 aquarium volumes per day. The test area was illuminated with a combination of Grow-Lux[R] and soft white fluorescent bulbs at an intensity of 30-60 footcandles at the solution's surface. Lighting was maintained on a photoperiod of 16 hours light, 8 hours darkness.

The dissolved oxygen concentration and temperature of the test solutions were monitored on every weekday within one replicate aquarium of each treatment level and controls. Total hardness, alkalinity, specific conductance and pH of the test solutions were monitored weekly in one aquarium from each treatment and controls.

Solvent concentrations are presented as nominal values since solvent concentrations are rarely, if ever, analytically determined during aquatic toxicity testing. Therefore, nominal concentrations are of greatest importance for comparative purposes. Daily diluter function determinations and stock solution usage confirmed that the correct volumes of solvent were consistently being automatically delivered to the test vessels throughout the exposures (Table 1). During flow-through tests performed with

dimethyl formamide and triethylene glycol at 19°C, Cardwell et al. (1978) found that mean measured concentrations remained very close to the nominal concentrations (96% of nominal with dimethyl formamide, 110% of nominal for triethylene glycol).

Twenty daphnids were impartially assigned to each test aquarium at the initiation of each test. Adult survival was determined weekly and determinations of offspring production were made on weekdays between days 7 and 28. The offspring were removed, counted with a Fisher Count-All® Model 600 particle counter (LeBlanc, 1979) and discarded. The tests were terminated after 28 days exposure.

Daphnids were fed a combination of Rangen Salmon starter fish food suspension (5 mg/L) and unicellular green algae, Selenastrum capricornutum (1-2 x 10^7 cells/mL). The food was introduced into each aquarium at a rate of 2.5 mL of fish food suspension and 1.0 mL of algae suspension three times daily on weekdays and once daily on weekends.

Survival and reproductive data derived during the chronic toxicity test were subjected to analysis of variance. If significant (P=0.05) differences between treatments were observed, the Dunnett's procedure was used to determine which treatments, if any, varied from the controls. Results of the statistical analyses were used to estimate the maximum acceptable toxicant concentration (MATC). The MATC is defined as the maximum concentration of solvent

which would not elicit an adverse response by *D. magna* based on the results of the chronic toxicity test.

RESULTS

Acute Toxicity Tests

Results of acute toxicity tests with acetone, dimethyl formamide and triethylene glycol are presented in Tables 2, 3 and 4, respectively. All three solvents had a low acute toxicity to *D. magna* with dimethyl formamide being the most toxic solvent and acetone being the least toxic. The 24- and 48-hour LC50 values and corresponding 95% confidence intervals are presented in Table 5. Dissolved oxygen concentrations and pH's measured during the exposures are presented in Table 6. These parameters remained within acceptable ranges for the survival of *D. magna*. The pH of test solutions containing triethylene glycol was the only parameter appreciably altered relative to the concentration of solvent.

Based on these results, the nominal concentrations selected to perform the chronic toxicity tests were: 11000, 5500, 2800, 1400 and 690 μL/L for acetone; 10000, 5000, 2500, 1200 and 600 μL/L for dimethyl formamide; 22000, 11000, 5500, 2800 and 1400 μL/L for triethylene glycol.

Chronic Toxicity Tests

Results of the water quality analysis of test solutions during the chronic toxicity tests with acetone, dimethyl formamide and triethylene glycol are presented in Tables 7, 8 and 9, respectively. Solutions containing high concentrations of the three solvents had lower dissolved oxygen levels than the controls. The reduction in dissolved oxygen concentrations was presumably due to the proliferation of microbial growth utilizing the solvent as a carbon source. Lower pH values were also measured in the high concentrations of dimethyl formamide and triethylene glycol. Temperature, total hardness, total alkalinity and specific conductance of the test solution varied minimally within and among tests.

Acetone

Survival of daphnids was significantly reduced after 7 days exposure to 11000, 5500 and 2800 μL/L acetone (Table 10). All daphnids exposed to 11000 μL/L died within the first four days of exposure. Daphnids surviving the exposure to 5500 μL/L on day 7 appeared smaller than the daphnids exposed to acetone concentrations less than 5500 μL/L and controls. Survival was relatively constant at all treatment levels beyond 7 days exposure.

Surviving daphnids exposed to 5500 μL/L acetone failed to reproduce for the duration of the 28-day exposure. Daphnids exposed

to 2800 μL/L experienced a significant lag in fecundity during the initial days of offspring production (days 9 and 10). These daphnids subsequently recovered in reproductive capacity and produced offspring in numbers comparable to the controls (Table 11, Figure 2). The number of offspring produced by daphnids exposed to 690 and 1400 μL/L acetone was comparable to the number of offspring produced by control daphnids for the duration of the exposure.

Based on the reduced survival of daphnids exposed to 2800 μL/L acetone, the MATC of this solvent for D. magna was >1400<2800 μL/L.

Dimethyl Formamide

Survival of daphnids exposed to dimethyl formamide concentrations of 5000 and 10000 μL/L was significantly reduced after 7 days exposure (Table 12). No daphnids survived the initial 7 days exposure to 10000 μL/L. Surviving daphnids exposed to 5000 μL/L dimethyl formamide for 7 days were appreciably smaller than the daphnids exposed to dimethyl formamide concentrations less than 5000 μL/L and controls. Mortality of daphnids exposed to 5000 μL/L dimethyl formamide continued throughout the exposure with no daphnids surviving on test day 28. Survival of daphnids exposed to 2500 μL/L dimethyl formamide was comparable to the controls during the initial two weeks of exposure, but was significantly reduced by test day 21. Survival of daphnids exposed to 600 and

1200 μL/L dimethyl formamide was unaffected for the duration of the exposure.

Surviving daphnids exposed to 5000 μL/L dimethyl formamide failed to reproduce throughout the exposure (Table 13, Figure 3). Daphnids exposed to 2500 μL/L dimethyl formamide produced significantly fewer offpsring between days 7 and 21. Although these daphnids continued to produce fewer offspring during the final week of exposure, this reduction was not statistically significant due to increased variability within replicate treatments.

Based on the reduced survival and offspring production of daphnids exposed to 2500 μL/L dimethyl formamide, the MATC of this solvent for _D. magna_ was >1200<2500 μL/L.

Triethylene Glycol

Survival of daphnids was not significantly affected by exposure to triethylene glycol concentrations as high as 22000 μL/L for 7 days (Table 14). However, daphnids exposed to 22000 μL/L during the initial 7 days of exposure appeared smaller than the daphnids exposed to triethylene glycol concentrations less than 22000 μL/L and controls. All daphnids exposed to 22000 μL/L died between test days 7 and 14. Survival of daphnids exposed to 11000 μL/L was unaffected through the initial 14 days of exposure but was significantly reduced by test day 21. Survival

of daphnids exposed to 1400, 2800 and 5500 μL/L triethylene glycol was unaffected for the duration of the exposure.

Surviving daphnids exposed to 22000 μL/L triethylene glycol failed to reproduce (Table 15, Figure 4). Daphnids exposed to 11000 μL/L triethylene glycol produced offspring in numbers comparable to the controls through test day 13, but subsequently produced significantly fewer offspring relative to the controls. The number of offspring produced by daphnids exposed to 1400, 2800 and 5500 μL/L triethylene glycol was comparable to the number of offspring produced by control daphnids for the duration of the exposure.

Based on the reduced survival and offspring production of daphnids exposed to 11000 μL/L triethylene glycol, the MATC for this solvent was >5500<11000 μL/L.

DISCUSSION

Results of this study clearly demonstrate that acetone, dimethyl formamide and triethylene glycol have low toxicity to *D. magna*. The order of toxicity was not consistent between acute and chronic toxicity tests for these materials. Dimethyl formamide was the most acutely toxic solvent tested while acetone was the least. Dimethyl formamide was also the most chronically toxic solvent tested; however, triethylene glycol was considerably less toxic

than both dimethyl formamide and acetone during the chronic exposure.

Acetone exhibited a minimal increase in toxicity after 24 hours during the acute toxicity test (Figure 5). This was initially presumed to be due to the loss of acetone from the solutions through volatilization. However, a similar lack of cumulative toxicity was observed during the chronic exposure (Figure 6). Test solutions presumably maintained consistent levels of acetone during the 28-day exposure as indicated by the proper diluter function, cloudiness of the solution and odor relative to the acetone concentration. Triethylene glycol and dimethyl formamide exhibited cumulative toxicity throughout the 28-day exposure. The MATC's defined in these tests could have been determined in 21 rather than 28 days, lending support to the recommended duration time of 21 days for daphnid chronic toxicity tests proposed by Adams (1981) as a standard practice. However, when cumulative toxicity is indicated, as with dimethyl formamide and triethylene glycol, it would be advantageous to extend the exposure until toxicity related mortalities cease.

Results of these chronic toxicity tests are inconsistent with the results generated by Cardwell et al. (1978) with brook trout and fathead minnows. Results presented herein suggest that *D. magna* are tolerant to the effects of dimethyl formamide and triethylene glycol, as compared to rainbow trout and fathead minnows, by

roughly two orders of magnitude. It appears that the differences in toxicity are not due to individual species sensitivity but rather due to extraneous factors caused by the presence of the solvents in water, specifically - microbial proliferation. During the fish exposures, uncontrolled growth of the bacterium *Sphaerotilus* sp. was noted. These organisms were implicated in the observed toxicity of the solvents by growing on the eggs, thereby possibly reducing oxygen assimilation and metabolism or by clogging the water exchange screens on the egg incubation cups thereby lowering the dissolved oxygen available to the developing embryos. The solvents also served as a carbon substrate for microbial proliferation during our tests as noted by the accumulation of "slime" on the aquaria walls and drainage screens. These growths never posed difficulties during the daphnid exposures since daphnids readily fed on protists and the test vessels were cleaned regularly.

In conclusion, triethylene glycol was the least chronically toxic of the three solvents tested and is recommended as the primary choice when selecting a carrier solvent for toxicity tests with *D. magna*. All three solvents are sufficiently low in toxicity to suggest that the recommended usage limits (500 μL/L during acute toxicity tests, 100 μL/L during long-term toxicity tests) are adequate for the prevention of solvent related toxicity to *D. magna*.

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TABLE 1. MEAN (STANDARD DEVIATION) DILUTER CYCLE RATE AND ACTUAL VS. THEORETICAL SOLVENT STOCK SOLUTION CONSUMPTION PER 24-HOUR INTERVAL.

Test Material	Diluter cycle rate/ 24 hours	Actual stock consumption/ 24 hours (L)	Theoretical stock consumption/ 24 hours (L)
Acetone	196(19)	1.2(0.1)	1.1(0.1)
Dimethyl formamide	158(17)	1.0(0.1)	1.0(0.1)
Triethylene glycol	175(3)	1.7(0.1)	1.6(0.0)

TABLE 2. CONCENTRATIONS TESTED AND CORRESPONDING OBSERVED PERCENTAGE MORTALITIES FOR THE WATER FLEA (*Daphnia magna*) EXPOSED TO ACETONE.

Nominal concentration (μL/L)	24 hour				48 hour			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
50,000	60	60	80	67[a]	60	80	80	73[a,c]
30,000	0	0	20	7[b]	20	20	40	27
18,000	0	0	20	7	0	0	20	7
11,000	0	0	0	0	0	0	0	0
6,500	0	0	0	0	0	0	0	0
3,900	0	0	0	0	0	0	0	0
2,300	0	0	0	0	0	0	0	0
control	0	0	0	0	0	0	0	0

[a] Several daphnids had flared carapaces.

[b] One daphnid was lethargic.

[c] Several daphnids were lethargic.

TABLE 3. CONCENTRATIONS TESTED AND CORRESPONDING OBSERVED PERCENTAGE MORTALITIES FOR THE WATER FLEA (*Daphnia magna*) EXPOSED TO DIMETHYL FORMAMIDE.

Nominal concentration (μL/L)	Percentage mortality							
	24 hour				48 hour			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
60,000	100	100	100	100	100	100	100	100
36,000	100	100	100	100	100	100	100	100
22,000	100	100	100	100	100	100	100	100
13,000	0	0	40	13[a,b]	20	40	80	47[a]
7,800	0	0	0	0[a]	0	0	0	0[a,b]
4,700	0	0	0	0	0	20	0	7
control	0	0	0	0	0	0	0	0

[a] Several daphnids were lethargic.

[b] Several daphnids were at the surface of the test solution.

TABLE 4. CONCENTRATIONS TESTED AND CORRESPONDING OBSERVED PERCENTAGE MORTALITIES FOR THE WATER FLEA (*Daphnia magna*) EXPOSED TO TRIETHYLENE GLYCOL.

Nominal concentration (μL/L)	Percentage mortality							
	24 hour				48 hour			
	A	B	C	$\bar{x}$	A	B	C	$\bar{x}$
100,000	100	100	100	100	100	100	100	100
60,000	40	80	60	60	100	100	100	100
36,000	0	0	0	0	40	0	20	20
22,000	0	0	0	0	0	20	0	7
13,000	0	0	20	7	0	0	20	7
7,800	0	0	20	7	0	0	20	7
control	0	0	0	0	0	0	0	0

TABLE 5. ACUTE TOXICITIES OF ACETONE, DIMETHYL FORMAMIDE AND TRIETHYLENE GLYCOL TO THE WATER FLEA (*Daphnia magna*).

Test Material	LC50 (95% confidence interval) (μL/L) 24 hour	48 hour
Acetone	44000 (38000-54000)	39000 (31000-53000)
Dimethyl formamide	17000 (13000-22000)	13000 (10000-16000)
Triethylene glycol	58000 (50000-68000)	35000 (28000-46000)

TABLE 6. WATER QUALITY OF TEST SOLUTIONS DURING THE ACUTE TOXICITY TESTS WITH THREE ORGANIC SOLVENTS AND THE WATER FLEA (*Daphnia magna*).

Test material	Parameter	Nominal concentration (μL/L)	0-hour	48-hour
acetone	dissolved oxygen (mg/L)	50000	8.4(93)[a]	8.2(91)
		11000	8.7(97)	8.3(92)
		2300	8.8(98)	8.4(93)
		control	8.5(94)	8.3(92)
	pH	50000	8.3	8.2
		11000	8.2	8.2
		2300	8.2	8.2
		control	8.2	8.2
dimethyl formamide	dissolved oxygen (mg/L)	60000	8.5(94)	8.8(98)
		22000	8.5(94)	8.8(98)
		4700	8.4(93)	8.7(97)
		control	8.6(96)	8.6(96)
	pH	60000	8.4	8.4
		22000	8.3	8.3
		4700	8.2	8.3
		control	8.2	8.2
triethylene glycol	dissolved oxygen (mg/L)	100000	8.2(91)	8.7(97)
		36000	8.7(97)	8.8(98)
		7800	8.5(94)	8.6(96)
		control	8.6(96)	8.6(96)
	pH	100000	7.3	7.4
		36000	7.8	7.9
		7800	8.1	8.2
		control	8.2	8.2

[a]% of saturation

TABLE 7. WATER QUALITY ANALYSIS OF TEST SOLUTIONS DURING THE CHRONIC EXPOSURE OF THE WATER FLEA (*Daphnia magna*) TO ACETONE.

	Mean (standard deviation)					
Nominal concentration (µL/L)	Dissolved oxygen[a] (mg/L)	Temperature[a] (°C)	Total hardness[b] (mg/L $CaCO_3$)	Total alkalinity[b] (mg/L $CaCO_3$)	Specific conductance[b] (µmhos/cm)	pH range[b]
11,000	7.7(0.6)	21(0)	180(0)	130(0)	500(0)	7.7-7.9
5,500	7.3(1.6)	21(0)	170(0)	130(0)	500(0)	7.2-7.9
2,800	8.2(0.5)	21(0)	180(0)	130(0)	500(0)	7.8-7.9
1,400	7.9(1.1)	21(0)	180(0)	130(0)	500(0)	7.7-7.9
690	8.1(0.6)	21(0)	180(0)	130(0)	500(0)	7.7-7.9
control	8.4(0.5)	21(0)	180(0)	130(0)	500(0)	7.8-7.9

[a] n=19

[b] n=5

TABLE 8. WATER QUALITY ANALYSIS OF TEST SOLUTIONS DURING THE CHRONIC EXPOSURE OF THE WATER FLEA (*Daphnia magna*) TO DIMETHYL FORMAMIDE.

	Mean (standard deviation)					
Nominal concentration (μL/L)	Dissolved oxygen[a] (mg/L)	Temperature[a] (°C)	Total hardness[b] (mg/L $CaCO_3$)	Total alkalinity[b] (mg/L $CaCO_3$)	Specific conductance[b] (μmhos/cm)	pH range[b]
10,000	6.2(1.4)	21(1)	170(0)	130(0)	520(40)	7.5-8.3
5,000	7.6(0.8)	21(1)	170(0)	130(0)	520(40)	7.8-8.3
2,500	7.9(1.3)	21(1)	170(0)	130(0)	520(40)	7.8-8.1
1,200	7.7(1.5)	21(1)	170(0)	130(0)	500(0)	7.8-7.9
600	7.6(1.4)	21(1)	170(0)	130(0)	500(0)	7.8-7.9
control	8.4(0.5)	21(1)	170(0)	130(0)	500(0)	7.8-7.9

[a] n=18

[b] n=5

TABLE 9. WATER QUALITY ANALYSIS OF TEST SOLUTIONS DURING THE CHRONIC EXPOSURE OF THE WATER FLEA (*Daphnia magna*) TO TRIETHYLENE GLYCOL.

	Mean (standard deviation)					
Nominal concentration (µL/L)	Dissolved oxygen[a] (mg/L)	Temperature[a] (°C)	Total hardness[b] (mg/L $CaCO_3$)	Total alkalinity[b] (mg/L $CaCO_3$)	Specific conductance[b] (µmhos/cm)	pH range[b]
22,000	6.9(1.2)	21(0)	160(10)	120(10)	460(50)	7.3-7.7
11,000	7.3(1.0)	21(0)	160(20)	120(10)	500(0)	7.6-7.8
5,500	7.5(0.9)	21(0)	160(20)	120(10)	500(0)	7.7-7.9
2,800	7.7(1.0)	21(0)	160(20)	130(10)	500(0)	7.7-8.0
1,400	7.7(0.9)	21(0)	160(10)	120(10)	500(0)	7.9-8.0
control	8.2(0.7)	21(0)	160(10)	120(10)	500(0)	7.8-8.0

[a] n=20

[b] n=5

TABLE 10. WEEKLY MEAN (STANDARD DEVIATION) PERCENTAGE SURVIVAL OF THE WATER FLEA (*Daphnia magna*) DURING CHRONIC EXPOSURE TO CONCENTRATIONS OF ACETONE.

Nominal concentration (μL/L)	Day/ Percentage survival 7	14	21	28
11,000	0(0)[a]	0(0)[a]	0(0)[a]	0(0)[a]
5,500	5(4)[a]	2(3)[a]	2(3)[a]	2(3)[a]
2,800	68(10)[a]	66(11)[a]	59(16)[a]	55(19)[a]
1,400	95(6)	95(6)	93(6)	89(5)
690	98(5)	98(5)	95(7)	94(6)
control	94(5)	91(2)	91(2)	90(0)

[a]Significantly (p=0.05) different from control.

TABLE 11. MEAN (STANDARD DEVIATION) CUMULATIVE OFFSPRING PRODUCED PER FEMALE *D. magna* DURING 28 DAYS OF EXPOSURE TO ACETONE.

Nominal concentration (µL/L)	Day														
	8	9	10	11	14	15	16	17	18	21	22	23	24	25	28
11,000	-[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5,500	0(0)	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]	0(0)[b]
2,800	0(0)	0(1)[b]	1(1)[b]	6(2)	26(6)	34(6)	39(7)	48(10)	54(9)	60(11)	63(11)	68(12)	78(18)	82(21)	93(19)
1,400	0(0)	2(0)	4(1)	6(1)	28(5)	39(5)	43(4)	50(6)	58(6)	79(9)	81(9)	92(10)	100(13)	104(12)	113(12)
690	1(0)	2(1)	4(1)	6(2)	25(3)	35(6)	39(4)	47(4)	56(6)	76(5)	81(4)	91(6)	97(7)	100(9)	111(12)
control	0(1)	3(3)	4(2)	7(3)	24(6)	30(5)	34(4)	42(6)	47(6)	67(6)	70(6)	79(7)	85(7)	88(6)	98(6)

[a] On test day 4 no surviving daphnids remained at this treatment level.

[b] Significantly ($p=0.05$) different from control.

TABLE 12. WEEKLY MEAN (STANDARD DEVIATION) PERCENTAGE SURVIVAL OF THE WATER FLEA (*Daphnia magna*) DURING CHRONIC EXPOSURE TO CONCENTRATIONS OF DIMETHYL FORMAMIDE.

Nominal concentration (μL/L)	Day/ Percentage survival 7	14	21	28
10,000	0(0)[a]	0(0)[a]	0(0)[a]	0(0)[a]
5,000	78(29)[a]	28(43)[a]	15(30)[a]	0(0)[a]
2,500	100(0)	86(10)	61(30)[a]	39(45)[a]
1,200	100(0)	98(3)	94(5)	90(4)
600	100(0)	94(12)	92(12)	86(8)
control	100(0)	99(2)	98(3)	94(2)

[a]Significantly ($p=0.05$) different from control.

TABLE 13. MEAN (STANDARD DEVIATION) CUMULATIVE OFFSPRING PRODUCED PER FEMALE D. *magna* DURING 28 DAYS OF EXPOSURE TO DIMETHYL FORMAMIDE.

Nominal concentration (µL/L)	Day 7	8	10	13	14	15	16	17	20	21	22	23	24	27	28
10,000	-[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5,000	0(0)	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	0(0)[c]	-[b]	-
2,500	0(0)	0(0)[c]	2(1)[c]	17(3)[c]	20(2)[c]	22(2)[c]	25(4)[c]	26(4)[c]	42(7)[c]	51(5)[c]	63(5)	64(2)	68(1)	74(2)	80(5)
1,200	1(1)	4(1)	8(1)	28(6)	32(8)	40(12)	43(12)	45(12)	63(11)	76(14)	80(14)	87(15)	95(17)	104(22)	109(23)
600	2(1)	6(4)	10(2)	32(10)	40(16)	47(16)	50(17)	54(17)	74(21)	87(22)	92(24)	100(29)	105(30)	115(37)	120(38)
control	1(0)	5(2)	8(2)	32(6)	38(7)	50(7)	54(10)	56(10)	73(13)	87(13)	91(15)	97(15)	100(14)	110(14)	115(15)

[a] On test day 7 no surviving daphnids remained at this treatment level.

[b] On test day 27 no surviving daphnids remained at this treatment level.

[c] Significantly ($p=0.05$) different from control.

TABLE 14. WEEKLY MEAN (STANDARD DEVIATION) PERCENTAGE SURVIVAL OF THE WATER FLEA (*Daphnia magna*) DURING CHRONIC EXPOSURE TO CONCENTRATIONS OF TRIETHYLENE GLYCOL.

Nominal concentration (μL/L)	Percentage survival Day/ 7	14	21	28
22,000	91(12)	0(0)[a]	0(0)[a]	0(0)[a]
11,000	98(3)	86(15)	55(38)[a]	29(31)[a]
5,500	100(0)	92(3)	90(0)	86(2)
2,800	100(0)	94(6)	88(5)	86(2)
1,400	100(0)	98(3)	91(8)	89(5)
control	99(2)	96(2)	94(2)	91(2)

[a]Significantly (p=0.05) different from control.

TABLE 15. MEAN (STANDARD DEVIATION) CUMULATIVE OFFSPRING PRODUCED PER FEMALE *D. magna* DURING 28 DAYS OF EXPOSURE TO TRIETHYLENE GLYCOL.

Nominal concentration (µL/L)	Day 9	12	13	14	15	16	20	21	22	23	26	27	28
22,000	0(0)[b]	0(0)[b]	0(0)[b]	-[a]	-	-	-	-	-	-	-	-	-
11,000	3(2)	14(1)	14(1)	17(2)[b]	21(3)[b]	22(4)[b]	30(7)[b]	36(11)[b]	38(12)[b]	39(12)[b]	44(17)[b]	47(19)[b]	50(20)[b]
5,500	4(1)	16(2)	19(2)	26(5)	33(4)	37(4)	56(4)	68(7)	71(7)	80(5)	96(6)	104(8)	109(6)
2,800	3(2)	19(3)	22(3)	30(1)	40(4)	44(5)	62(5)	74(8)	77(8)	87(10)	102(11)	111(14)	116(13)
1,400	4(2)	16(5)	19(3)	27(2)	36(5)	39(4)	55(4)	66(5)	72(5)	80(7)	99(12)	106(13)	112(14)
control	3(1)	17(5)	20(6)	30(6)	36(8)	40(8)	57(11)	69(11)	73(10)	81(10)	95(10)	104(11)	110(10)

[a] No surviving daphnids remained at this treatment level.

[b] Significantly ($p=0.05$) different from control.

Figure 1. Tube siphon delivery apparatus used to deliver the appropriate volume of solvent to the mixing chamber of the diluter with each cycle.

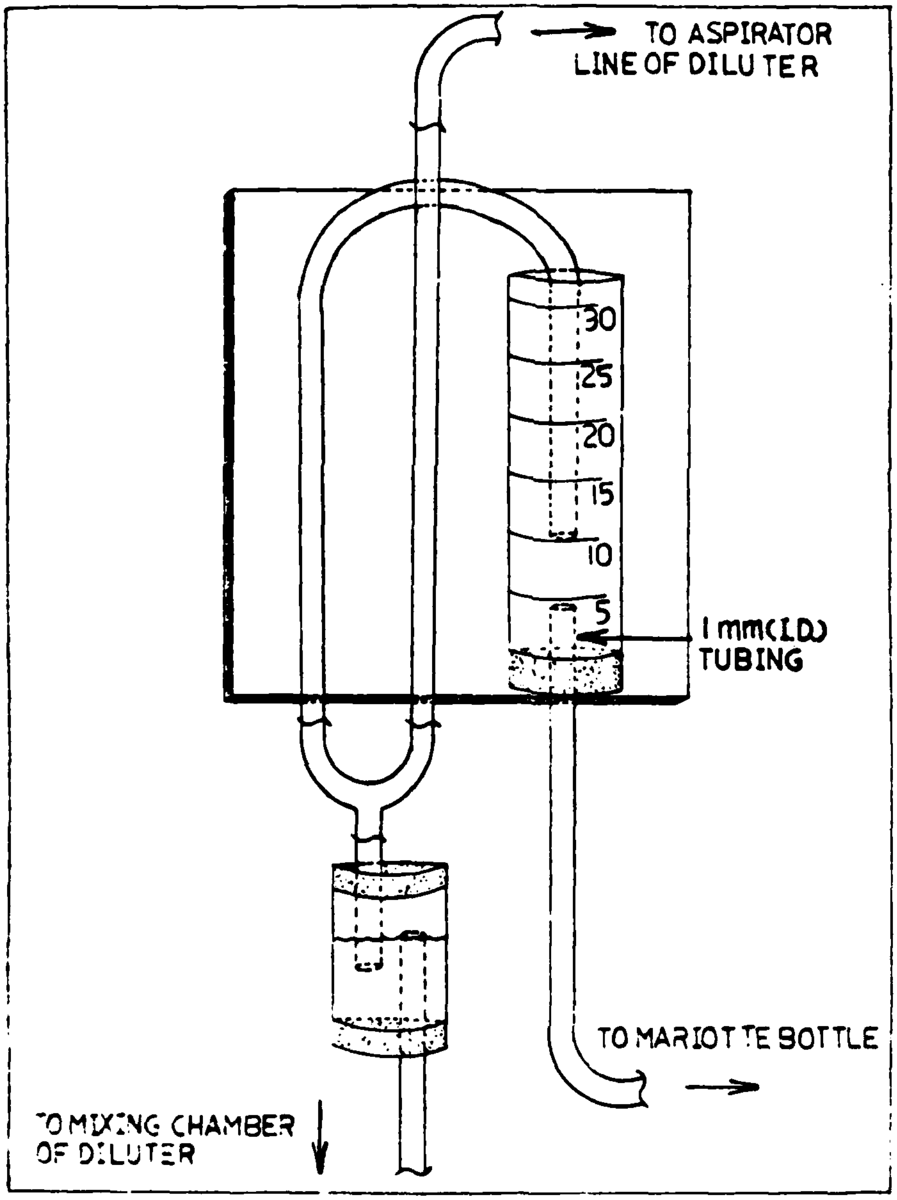


Figure 2. Cumulative number of offspring produced per female D. magna exposed to concentrations of acetone for 28 days.

○ = control, 690 µL/L = □, 1400 µL/L = ◇, 2800 µL/L = △, ● = 5500 µL/L.

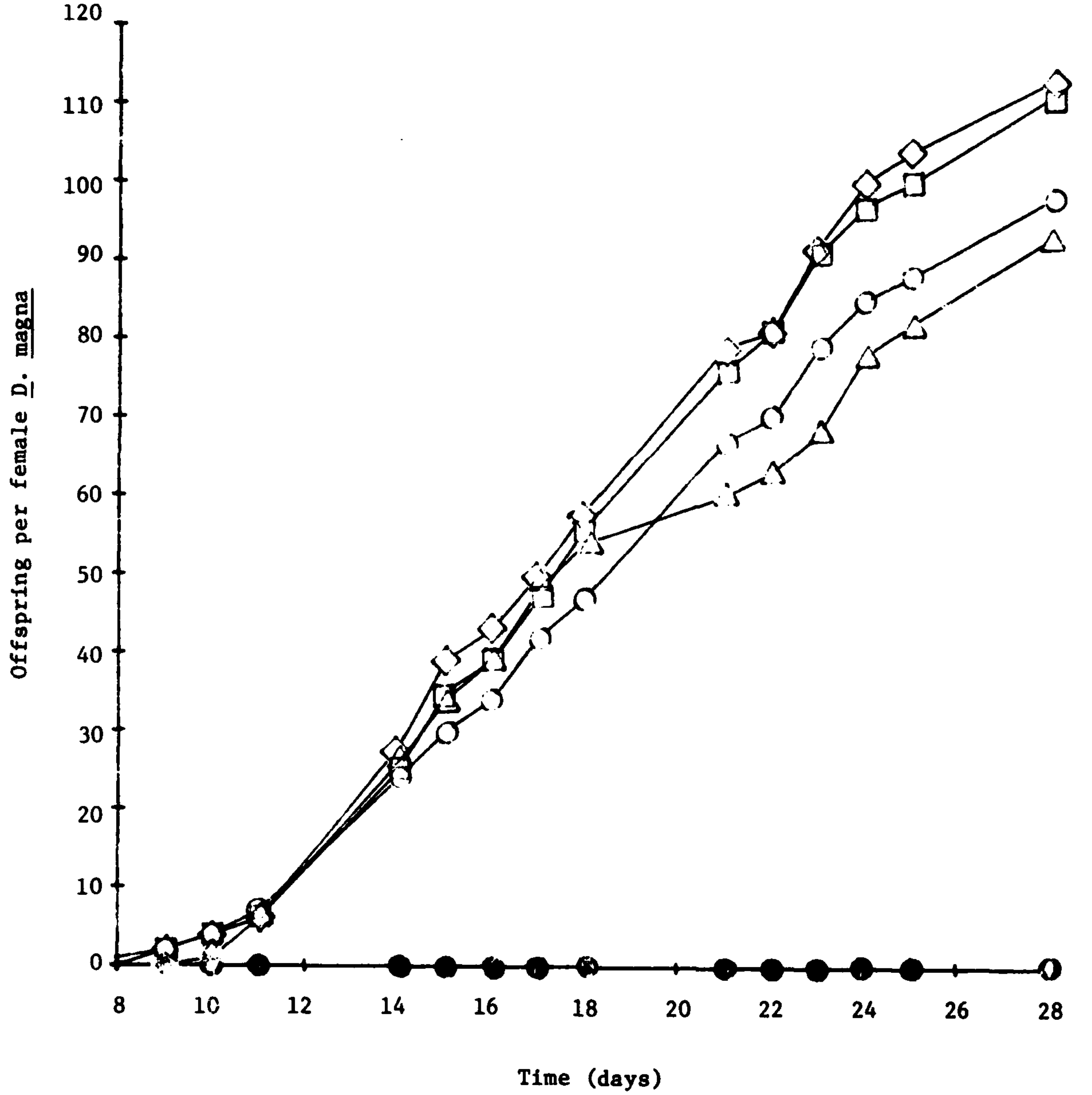


Figure 3. Cumulative number of offspring produced per female *D. magna* exposed to concentrations of dimethyl formamide for 28 days.

○ = control, □ = 600 μL/L, ◇ = 1200 μL/L, △ = 2500 μL/L, ● = 5000 μL/L.

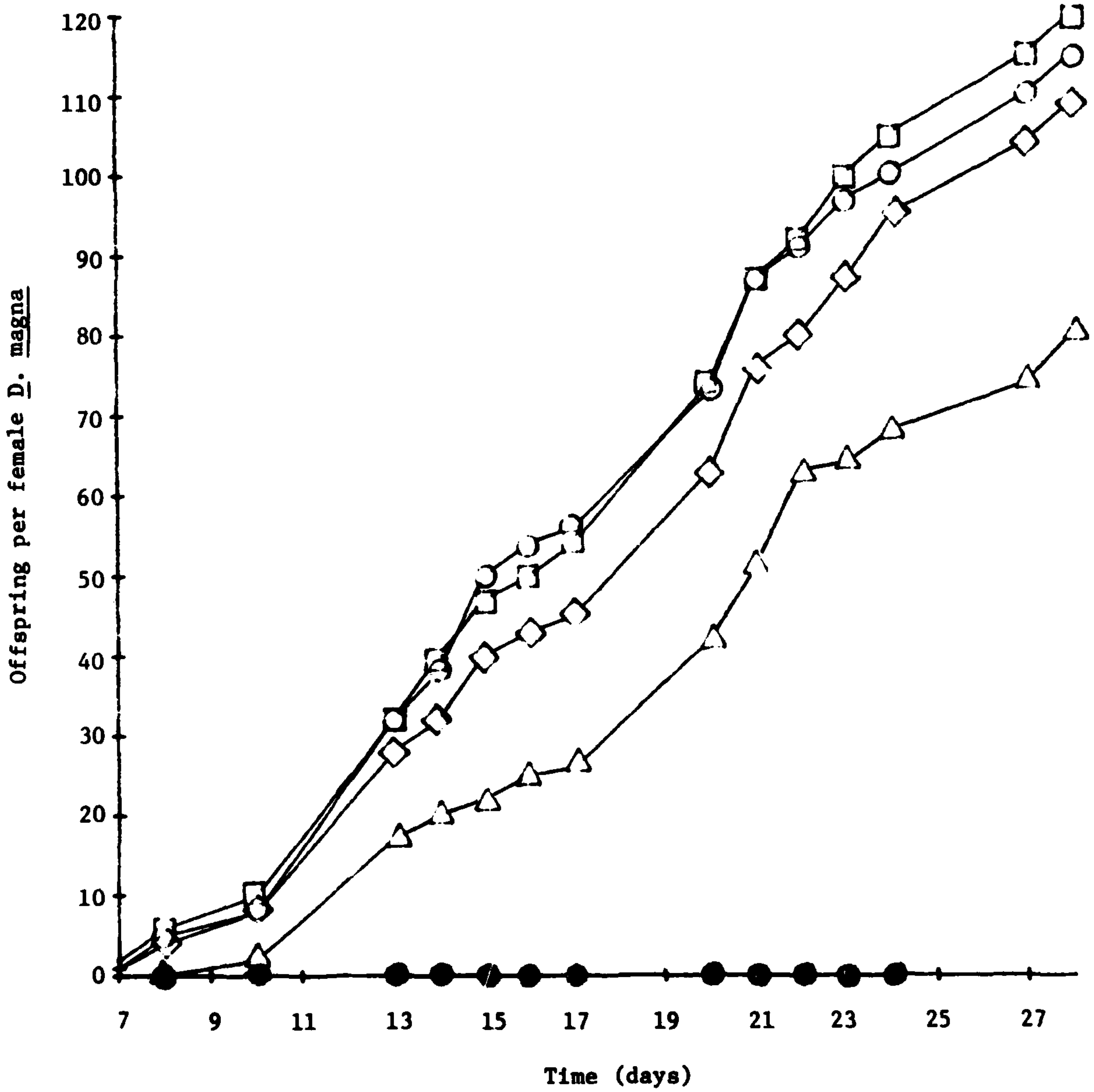


Figure 4. Cumulative number of offspring produced per female D. magna exposed to concentrations of triethylene glycol for 28 days.

○ = control, □ = 1400 µL/L, ◇ = 2800 µL/L, △ = 5500 µL/L, ● = 11000 µL/L, ■ = 22000 µL/L.

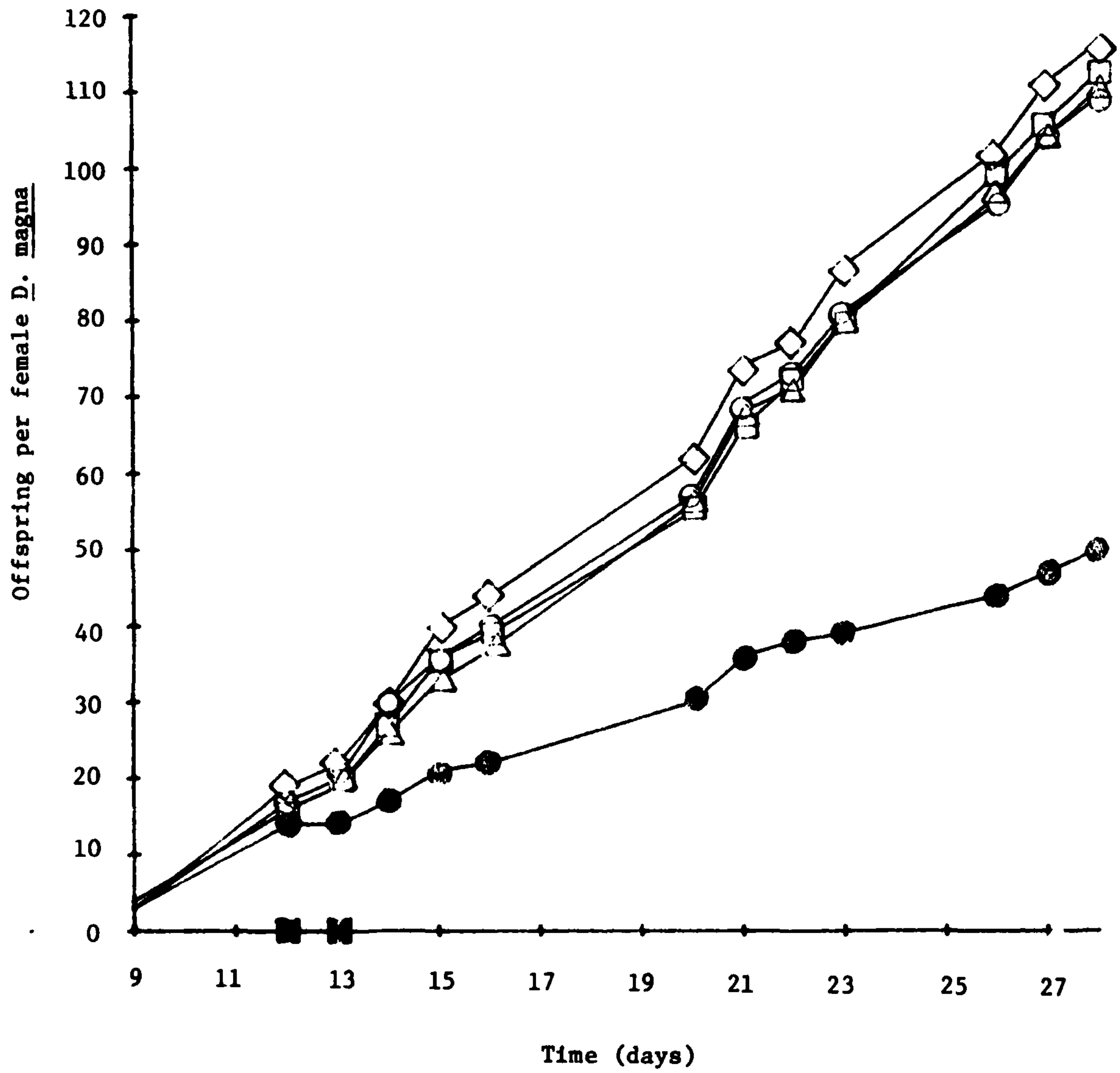


Figure 5. Acute toxicity, expressed as median lethal concentrations, of three organic solvents with *D. magna*.

■ = acetone, O = dimethyl formamide, ◊ = triethylene glycol

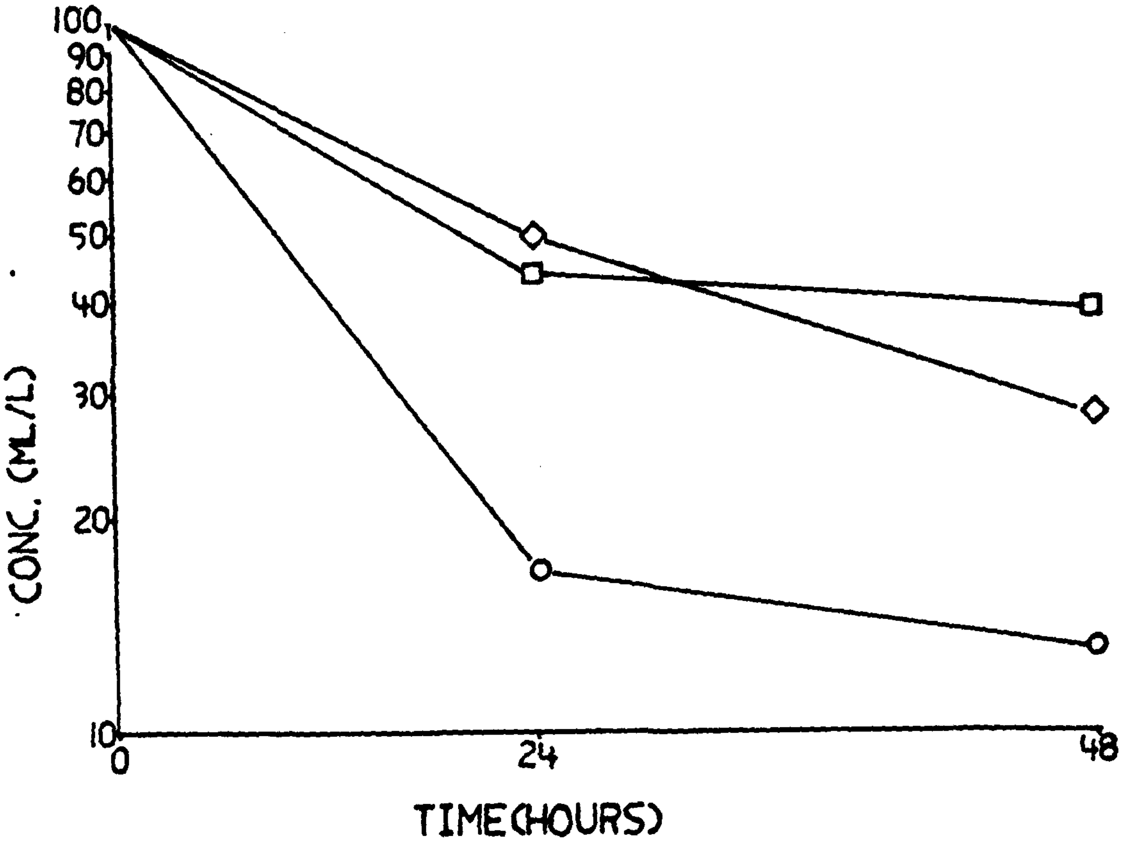
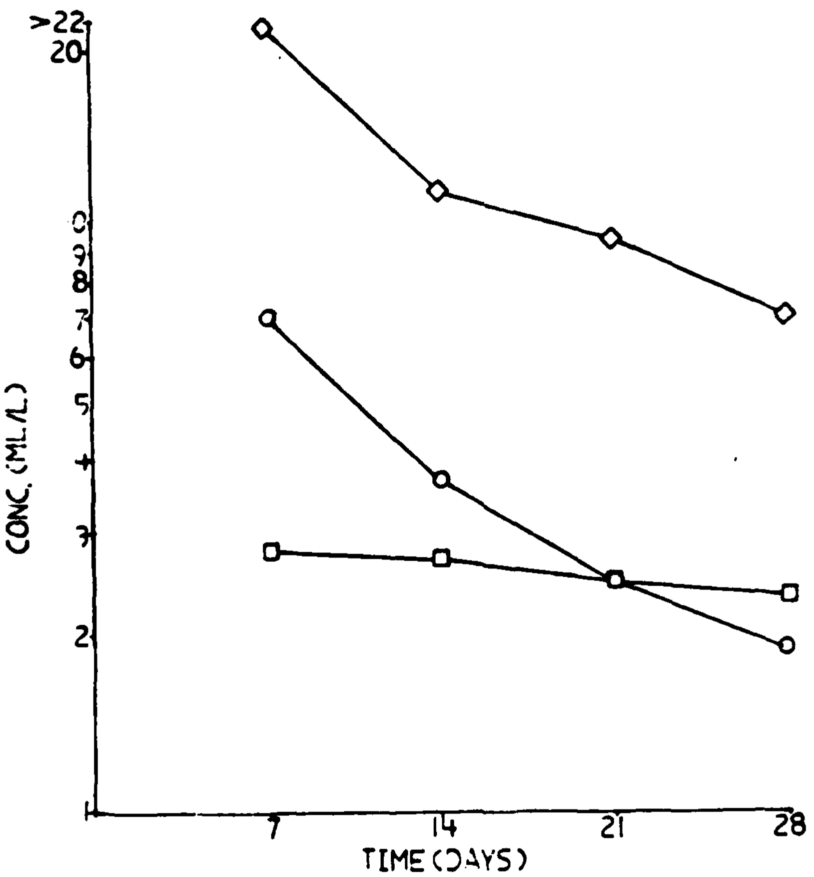


Figure 6. Survival, expressed as median lethal concentrations, of D. magna exposed to three organic solvents for 28 days.

□ = acetone, O = dimethyl formamide, ◊ = triethylene glycol



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